

## University of Rhode Island DigitalCommons@URI

---

Past Departments Faculty Publications

College of the Environment and Life Sciences

---

1983

# Relationship Between the Mouse Colonizing Ability of a Human Fecal *Escherichia coli* Strain and its Ability to Bind a Specific Mouse Colonic Mucous Gel Protein

Paul S. Cohen

University of Rhode Island, [pscohen@uri.edu](mailto:pscohen@uri.edu)

Richard Rossoll

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.uri.edu/cels\\_past\\_depts\\_facpubs](https://digitalcommons.uri.edu/cels_past_depts_facpubs)

### Terms of Use

All rights reserved under copyright.

---

### Citation/Publisher Attribution

Cohen, P. S., Rossoll, R., Cabelli, V. J., Yang, S.L., & Laux, D. C. (1983). Relationship Between the Mouse Colonizing Ability of a Human Fecal *Escherichia coli* Strain and its Ability to Bind a Specific Mouse Colonic Mucous Gel Protein. *Infect. Immun.*, 40(1), 62-69. Retrieved from <http://iai.asm.org/content/40/1/62>. Available at: <http://iai.asm.org/content/40/1/62>

This Article is brought to you for free and open access by the College of the Environment and Life Sciences at DigitalCommons@URI. It has been accepted for inclusion in Past Departments Faculty Publications by an authorized administrator of DigitalCommons@URI. For more information, please contact [digitalcommons@etal.uri.edu](mailto:digitalcommons@etal.uri.edu).

---

**Authors**

Paul S. Cohen, Richard Rossoll, Victor J. Cabelli, Shiao-Lien Yang, and David C. Laux

## Relationship Between the Mouse Colonizing Ability of a Human Fecal *Escherichia coli* Strain and Its Ability to Bind a Specific Mouse Colonic Mucous Gel Protein

PAUL S. COHEN,\* RICHARD ROSSOLL, VICTOR J. CABELLI, SHIAO-LIEN YANG, AND DAVID C. LAUX

University of Rhode Island, Department of Microbiology, Kingston, Rhode Island 02881

Received 28 September 1982/Accepted 27 December 1982

*Escherichia coli* F-18, isolated from the feces of a healthy human, is an excellent colonizer of the large intestines of streptomycin-treated CD-1 mice. *E. coli* F-18 Col<sup>-</sup>, a poor mouse colonizer relative to F-18, lacks a  $3 \times 10^7$ -dalton plasmid present in *E. coli* F-18. Both strains are human type A erythrocyte hemagglutination negative, have identical surface hydrophobicities, contain the same number of lipopolysaccharide molecules with the same O-side chain length, and have identical amounts of capsule. Differences between the two strains were observed. The relative amounts of specific outer membrane proteins differed, and *E. coli* F-18 was less motile than *E. coli* F-18 Col<sup>-</sup>. The abilities of the two strains to bind mouse large intestine mucous gel was also examined. Although each strain was able to use mucous gel as the sole source of carbon and nitrogen with equal ability, *E. coli* F-18 bound between two and three times more mucous gel than did *E. coli* F-18 Col<sup>-</sup>. Most of the difference in mucous gel binding ability of the two strains was accounted for by the greater ability of *E. coli* F-18 lipopolysaccharide to bind a single 26,000-dalton mucous gel protein. *E. coli* J5-3, a typical K-12 strain that is also a poor colonizer relative to *E. coli* F-18, was identical to *E. coli* F-18 Col<sup>-</sup> with respect to mucous gel binding ability.

In a number of instances, the initiation of bacterial infection has been shown to involve adhesion to mucosal surfaces. One striking example of such adhesion occurs in diseases involving enterotoxigenic strains of *Escherichia coli*. In the case of these organisms, plasmid-encoded cell surface factors such as the K88, K99, 987P, CFA/I, or CFA/II fimbrial antigens have been implicated in the adhesion process (7, 8, 12, 15, 23). Fimbriae appear to be required for enterotoxigenic *E. coli* adhesion to small intestine epithelial cells, and adhesion is required for enterotoxigenic *E. coli* establishment in the small intestine.

In contrast to the specific data available on small intestine adhesion by enterotoxigenic *E. coli*, relatively little is known regarding the mechanism by which nonenterotoxigenic strains of *E. coli* adhere to the mucosal surface of the large intestine. The large intestine is known to contain large numbers of *E. coli*, and these organisms have been observed in close association with the brush border cells of the human large intestine (9). Normally, the brush border epithelial cells of the large intestine are covered

by an outer glycoprotein layer (3) that is called the mucous gel. This material is glycoprotein in nature and represents a major component of the mucosal surface (1, 3). Although mucous gel is present in relatively large amounts, its role in *E. coli* adhesion to the large intestine remains largely unknown. In this regard, it is possible that mucous gel serves at least two functions. First, it might be a source of nutrients for *E. coli* growth; indeed, some enteric organisms have been shown to degrade hog mucin (11). Second, mucous gel might form a matrix to which *E. coli* strains bind as a first step in the colonization process.

In a recent report, we presented evidence which indicated that simple genetic changes (e.g., resistance to rifampicin, loss or gain of a plasmid) can decrease or increase the colonizing ability of a human *E. coli* strain in streptomycin-treated mice (21). One such strain, *E. coli* F-18, is about 10,000-fold better in terms of its ability to colonize the mouse large intestine than a derivative of *E. coli* F-18, *E. coli* F-18 Col<sup>-</sup>, or a K-12 strain, *E. coli* J5-3. In the present investigation, we have examined the surfaces of these

strains and used them to study the interaction between mucous gel and *E. coli* strains of various colonizing abilities.

## MATERIALS AND METHODS

**Bacteria.** *E. coli* F-18 was isolated in 1977 from the feces of a healthy University of Rhode Island student by the method of Dufour et al. (6) and was confirmed as *E. coli* by the API 20E series of biochemical tests (Analytab Products Inc., Plainview, N.Y.). *E. coli* F-18 produces a trypsin-sensitive colicin and contains seven plasmids. *E. coli* F-18 Col<sup>-</sup> contains six plasmids and does not make the F-18 colicin. Neither strain hemagglutinates human type A erythrocytes. Attempts were made at serotyping *E. coli* F-18 and *E. coli* F-18 Col<sup>-</sup> by R. Wilson, *E. coli* Reference Center, The Pennsylvania State University, University Park, Pa. The two strains contained untypable H, K, and O antigens. *E. coli* F-18 Col<sup>-</sup> was isolated in the following way. A streptomycin-resistant, nalidixic acid-resistant mutant of *E. coli* F-18 was mated with *E. coli* RS-2, which contains the R1drd19 plasmid (R1<sup>+</sup>), and an F-18 R1<sup>+</sup> strain was isolated. The F-18 R1<sup>+</sup> strain was cured of the R1drd19 plasmid with acriflavin. One of the cured clones, F-18 Col<sup>-</sup>, lost both a  $3 \times 10^7$ -dalton plasmid and the ability to make the F-18 colicin. *E. coli* J5-3, a K-12 strain that requires proline and methionine for growth (2), was obtained from G. M. Thorne, Tufts University School of Medicine, Boston, Mass. *E. coli* K-12(K88ab) contains the K88ab plasmid and was obtained from R. Wilson.

**Mouse colonization experiments.** Recently, we described a model of large intestine colonization that can be used to distinguish relative colonizing abilities of *E. coli* strains in mice (21). Briefly,  $10^{10}$  CFU of each of two genetically marked strains of *E. coli* in 1 ml of 10% (wt/vol) sucrose are fed to CD-1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) whose normal facultative flora has been reduced by the addition of streptomycin sulfate (5 g/liter) to their drinking water. Subsequent monitoring of the level of each strain present in the feces then provides a reliable estimate of how well one strain colonizes the large intestine relative to the second competing strain.

In the present investigation, spontaneous streptomycin-resistant (Str<sup>r</sup>), nalidixic acid resistant (Nal<sup>r</sup>), and rifampicin resistant (Rif<sup>r</sup>) mutants were isolated after plating on L agar containing the appropriate antibiotic. *E. coli* F-18 was used as its F-18 Str<sup>r</sup> Rif<sup>r</sup> double mutant, *E. coli* F-18 Col<sup>-</sup> was used as its F-18 Col<sup>-</sup> Str<sup>r</sup> Nal<sup>r</sup> double mutant, and *E. coli* J5-3 was used as its J5-3 Str<sup>r</sup> Nal<sup>r</sup> double mutant. Control experiments showed that the Nal<sup>r</sup> and Rif<sup>r</sup> mutations did not alter the colonizing abilities of the immediate Str<sup>r</sup> parents. The double mutants will be referred to as strains F-18, F-18 Col<sup>-</sup>, and J5-3, respectively.

**Media and antibiotics.** L broth was made as described by Revel (24). L agar is L broth containing 12 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) per liter. Streptomycin sulfate, nalidixic acid, and rifampicin were purchased from Sigma Chemical Co., St. Louis, Mo., and were used in agar plates at concentrations of 100, 50, and 50 µg/ml, respectively. Motility agar is L broth containing 3.5 g of Bacto-Agar per liter.

**Motility.** Strains to be tested for motility were

streaked on L agar and incubated for 18 h at 37°C. At that time, samples of each strain were transferred with a toothpick from L agar to motility agar, the plates were incubated for 6 h at 37°C, and the diameters of visible growth were measured (17).

**KDO measurements.** 2-Keto-3-deoxyoctonate (KDO) measurements on whole *E. coli* cells and on purified lipopolysaccharide (LPS) preparations were made by the method of Karkhanis et al. (16).

**Hexose measurements.** Hexose was measured by the indole method (5) with galactose as the standard.

**Protein measurements.** *E. coli* protein was measured by the Folin method (19).

**Capsule weights.** Capsule was purified from the supernatant obtained after sedimentation of *E. coli* LPS from the aqueous phase of a warm water-phenol extract (28) of 1 liter of *E. coli* cells. The aqueous phase was lyophilized, and the capsular polysaccharide obtained was weighed on a Mettler HE10 balance equipped with a Mettler BA28 digital readout. Capsule preparations contained less than 10% nucleic acid and less than 1% protein.

**Hydrophobic-interaction chromatography.** Retention of bacteria on phenyl-Sepharose columns was measured as described by Jann et al. (13).

***E. coli* outer membrane isolation.** Outer membrane preparations of *E. coli* were isolated by the method of Schnaitman (26), and were reductively methylated with [<sup>3</sup>H]formaldehyde by the method of Jentoft and Dearborn (14). <sup>3</sup>H-labeled vesicular stomatitis virus proteins (L, 190,000 daltons; G, 69,000 daltons, N, 50,000 daltons; M, 29,000 daltons) were used as standards.

***E. coli* LPS isolation.** *E. coli* LPS was purified by the warm phenol-water method of Westphal and Jann (28) and was stored in water at 4°C until use.

**Polyacrylamide gel electrophoresis.** Tritium-labeled proteins were separated on 10% polyacrylamide slab gels as described previously (20). Dried gels were fluorographed by the procedure of Laskey and Mills (18), and developed films, when necessary, were analyzed with a Quick Scan Flur-Vis densitometer.

**Mucous gel isolation.** One day before mucous gel extraction, CD-1 mice were given streptomycin sulfate (5 g/liter) in their drinking water to reduce the facultative flora in the large intestines. Mice were killed with chloroform, and their large intestines (excluding the caecum) were removed and placed in phosphate-buffered saline at room temperature. Each of the intestines was cut into three to four pieces, approximately 1.5 cm in length, and each of the pieces of large intestine was cleared of feces by squeezing with a rubber spatula. The pieces of large intestine were then transferred to fresh, room-temperature phosphate-buffered saline and each piece was opened lengthwise with a scalpel. The opened pieces were then transferred to fresh room-temperature phosphate-buffered saline again (intestine from seven mice in 6 ml of phosphate-buffered saline), the mucosa (consisting of epithelial cells and mucous gel) was scraped into the phosphate-buffered saline with a rubber spatula, and the scraped pieces of intestine were removed. The mucosal suspension was centrifuged (27,000 × g) at room temperature for 20 min to remove both the epithelial cells and any remaining fecal debris. The top 5 ml of viscous supernatant, which contained the mucous gel, was removed with a pipette and saved. The bottom 1 ml of the supernatant

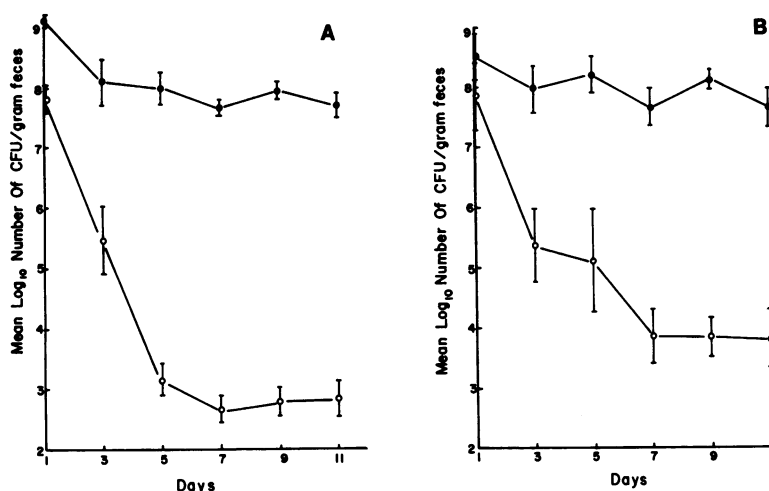


FIG. 1. Relative colonizing abilities of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 in streptomycin-treated mice. On day zero strains F-18 Str<sup>r</sup> Rif<sup>r</sup> and F-18 Col<sup>-</sup> Str<sup>r</sup> Nal<sup>r</sup> were fed to one set of six mice, and F-18 Str<sup>r</sup> Rif<sup>r</sup> and J5-3 Str<sup>r</sup> Nal<sup>r</sup> were fed to a second set of six mice. Fecal samples were spread on L agar plates containing 100  $\mu$ g of streptomycin sulfate per ml and either 50  $\mu$ g of rifampicin per ml or 50  $\mu$ g of naladixic acid per ml. Bars representing standard errors of the mean are presented for each data point. A, Symbols: (●) F-18, (○) F-18 Col<sup>-</sup>. B, Symbols: (●) F-18, (○) J5-3.

contained a loose, mucous-like pellet material, which upon recentrifugation was separated into a more firm pellet and a clear viscous supernatant. The two supernatants were combined and, depending on the preparation, contained between 6 and 8 mg of mucous gel protein per ml.

**Mucous gel labeling by reductive methylation.** After isolation, mucous gel proteins were reductively methylated with [<sup>3</sup>H]formaldehyde (2  $\mu$ mol/mg of protein, 65  $\mu$ Ci/ $\mu$ mol) by the method of Jentoft and Dearborn (14). After methylation, the <sup>3</sup>H-labeled mucous gel preparation was dialyzed extensively against HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-Hanks buffer, pH 7.4 (27), centrifuged at 100,000  $\times$  g for 2.25 h at 4°C to remove any particulate matter, and stored at 4°C. In control experiments, the relative amounts of individual mucous gel proteins determined by Coomassie blue staining of polyacrylamide gels were accurately reflected in fluorographs of individual <sup>3</sup>H-labeled mucous gel proteins on polyacrylamide gels.

**Mucous gel binding assay.** Unless otherwise stated, mucous gel binding to *E. coli* cells was performed as follows. *E. coli* was grown overnight in L broth in standing cultures at 37°C. Cultures were centrifuged, washed twice, and suspended in HEPES-Hanks buffer (pH 7.4) at  $6.0 \times 10^{10}$  cells per ml. <sup>3</sup>H-labeled mucous gel (72  $\mu$ g of protein,  $9.0 \times 10^5$  cpm) was mixed with  $2.4 \times 10^9$  *E. coli* cells in a total volume of 0.1 ml, and the mixture was incubated at 37°C for 1 h. Five milliliters of HEPES-Hanks buffer (pH 7.4) was then added to the reaction mixture, and the *E. coli* cells were centrifuged at 12,000  $\times$  g for 10 min at room temperature and washed in the same way twice in HEPES-Hanks buffer (pH 7.4). The washed *E. coli* cells were then suspended in 0.5 ml of HEPES-Hanks buffer (pH 7.4), and the bound mucous gel was counted in Aquasol (New England Nuclear Corp., Boston,

Mass.) in a Packard model 3000 Tri-Carb scintillation counter. Blank values (i.e., <sup>3</sup>H-labeled mucous gel incubated in the absence of *E. coli* cells) were always less than 500 cpm.

**Characteristics of the mucous gel binding assay.** (i) **Lysine involvement.** The  $\epsilon$  amino groups of lysine molecules are the only groups in protein molecules reductively methylated by formaldehyde (14). It was possible that the  $\epsilon$  amino groups of lysine were normally involved in mucous gel binding to *E. coli* which would invalidate the use of reductively methylated <sup>3</sup>H-labeled mucous gel for binding assays. If so, at <sup>3</sup>H-labeled mucous gel saturation the addition of an equal amount of nonradioactive, nonmethylated mucous gel would reduce <sup>3</sup>H-labeled mucous gel binding to *E. coli* cells by greater than 50%. This was not observed. At saturation (about 3.6 mg of <sup>3</sup>H-labeled mucous gel per ml reaction mixture containing  $2.4 \times 10^{10}$  *E. coli* cells), the addition of an equal amount of unmethylated mucous gel inhibited <sup>3</sup>H-labeled mucous gel binding to *E. coli* strains F-18, F-18 Col<sup>-</sup>, and J5-3 by exactly 50%, showing that  $\epsilon$  amino groups of lysine are not involved in mucous gel binding to *E. coli*.

(ii) **pH studies.** The pH of the large intestines of mammals has been reported to be neutral to alkaline (25). Mucous gel binding assays with *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 were performed in increments of 0.2 pH units in HEPES-Hanks buffer between pH 7.0 and pH 8.2. Between pH 7.0 and 7.6, no differences in the amounts of mucous gel bound by any one strain were observed (data not shown). Above pH 7.6, the blank values in the assays increased, presumably due to mucous gel precipitation, thereby destroying the sensitivity of the assay. All subsequent mucous gel binding assays were performed at pH 7.4.

(iii) **Kinetics of mucous gel binding.** Time course studies at 37°C with  $2.4 \times 10^9$  *E. coli* cells of either F-18, F-18 Col<sup>-</sup>, or J5-3 and 72  $\mu$ g of <sup>3</sup>H-labeled mucous

gel protein ( $9 \times 10^5$  cpm) in a total volume of 0.1 ml showed mucous gel binding to be linear for 10 to 15 min, after which binding essentially stopped. In all subsequent experiments reaction mixtures were incubated for 1 h.

(iv) **Temperature studies.** Mucous gel binding experiments were performed at 0 and 37°C. At 0°C the binding of mucous gel to strains F-18, F-18 Col<sup>-</sup>, and J5-3 was 32, 59, and 57%, respectively, of that observed at 37°C.

(v) **Hexose content of mucous gel.** Mucous gel was assayed for hexose content by the indole method (5) with galactose as the standard. The mucous gel contained an average of 5% (wt/wt) galactose equivalents relative to protein.

## RESULTS

**Relative colonizing abilities of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3.** The relative colonizing abilities of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3, were determined in the following way. Strains F-18, F-18 Col<sup>-</sup>, and J5-3 were each fed to streptomycin-treated CD-1 mice alone and in combinations of two strains at a time. The number of each strain per gram of feces was then determined at 48-h intervals for 11 days. Each of the strains colonized at levels of between  $10^7$  and  $10^8$  cells per g of feces when fed individually to mice, showing that the complex environment of the large intestine did not preferentially kill any of the three strains. However, when pairs of strains were fed to mice, *E. coli* F-18 colonized at a level 10,000-fold higher than did either F-18 Col<sup>-</sup> or J5-3 (Fig. 1). Strains F-18 Col<sup>-</sup> and J5-3 cocolonized mice at equal levels (data not shown).

**Surface properties of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3.** In an attempt to determine whether differences in surface properties could explain the greater colonizing ability of *E. coli* F-18 relative to *E. coli* F-18 Col<sup>-</sup> and *E. coli* J5-3, the following experiments were performed.

(i) **Hydrophobic interaction chromatography.** Human *E. coli* strains that contain type 1 pili are retained on phenyl-Sepharose columns in the presence of 1 mM ammonium acetate (13). Any

TABLE 2. Hexose/KDO ratio in purified *E. coli* LPS<sup>a</sup>

<i>E. coli</i> strain	Hexose/KDO ratio	
	Expt 1	Expt 2
F-18	3.88	3.41
F-18 Col <sup>-</sup>	4.08	3.11
J5-3	2.67	2.31

<sup>a</sup> Hexose and KDO were measured as described in the text.

strain that is not retained has no type 1 pili (13). *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 were retained to the extent of only 28, 30, and 24%, respectively. *E. coli* K-12(K88ab), a positive control (13), was retained to the extent of 76%. These results suggest that none of the strains contained type 1 pili.

(ii) **Amount of KDO per milligram of bacterial cell protein.** A single molecule of *E. coli* LPS core contains three molecules of KDO (22). The amount of KDO per milligram of bacterial cell protein is therefore in proportion to the number of LPS molecules per cell. By this criterion, *E. coli* F-18 and *E. coli* F-18 Col<sup>-</sup> had the same number of LPS molecules per cell (Table 1). *E. coli* J5-3 contained about 60% more LPS molecules per cell than either of the latter two strains (Table 1).

(iii) **Amount of LPS hexose per KDO molecule.** A comparison of the ratio of hexose to KDO in *E. coli* F-18 LPS to that of *E. coli* F-18 Col<sup>-</sup> LPS was taken as a reflection of the relative average lengths of the O-side chains of the two strains. The hexose/KDO ratios of the two strains were identical (Table 2). Also shown in Table 2 is the hexose/KDO ratio of the LPS of *E. coli* J5-3. *E. coli* J5-3 is a K-12 strain devoid of O-side chains (22), and therefore hexose in this instance is derived entirely from the core oligosaccharide.

(iv) **Capsule weights.** Capsule dry weight per milligram of bacterial protein was taken as a reflection of the amount of capsule per *E. coli* cell. *E. coli* strains F-18, F-18 Col<sup>-</sup>, and J5-3 all contained similar amounts of capsule per milligram of protein (Table 3).

(v) **Outer membrane proteins.** The outer membrane proteins of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 were compared by polyacrylamide gel electrophoresis. The molecular weights of the outer membrane proteins of *E. coli* F-18 and *E. coli* F-18 Col<sup>-</sup> were identical; however, the relative amounts of a few major proteins in the outer membranes of the two strains were different, e.g., the 39,500-dalton protein and the 36,000-dalton protein (Fig. 2). In contrast, *E. coli* J5-3 contained at least two proteins not present in the outer membranes of *E. coli* F-18 and *E. coli* F-18 Col<sup>-</sup>, a 35,800-dalton protein and a 23,000-

TABLE 1. *E. coli* KDO content

<i>E. coli</i> strain	KDO (μg/mg of total cell protein) <sup>a</sup>	
	Expt 1	Expt 2
F-18	6.85 <sup>b</sup>	7.46
F-18 Col <sup>-</sup>	7.26	5.69
J5-3	11.09	10.27

<sup>a</sup> Cultures (100 ml) were grown, washed, and suspended as described for the mucous gel binding assay (see text) before KDO and protein measurements.

<sup>b</sup> This culture contained 73.3 μg of KDO and 10.7 mg of bacterial protein. Protein contents in all cultures were similar.

TABLE 3. Amount of capsule per milligram of bacterial cell protein<sup>a</sup>

<i>E. coli</i> strain	Amt of capsule (mg) per mg of protein	
	Expt 1	Expt 2
F-18	0.13 <sup>b</sup>	0.12
F-18 Col <sup>-</sup>	0.12	0.10
J5-3	0.11	0.10

<sup>a</sup> Dry weight determinations were made of capsules extracted from 1-liter cultures (see text).

<sup>b</sup> In this case, the *E. coli* F-18 culture contained 16.1 mg of capsule and 127 mg of protein.

dalton protein (Fig. 2).

(vi) **Motility.** The relative motilities of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 were determined (see above). Typical 6-h diameters of visible growth at 37°C were as follows: F-18 Col<sup>-</sup>, 16 mm; J5-3, 10 mm; F-18, 6 mm. Clearly, the best colonizer, *E. coli* F-18, was the least motile. With this assay, visible growth diameters of nonmotile mutants are, at most, 2 mm (17).

**Mucous gel binding abilities of F-18, F-18 Col<sup>-</sup>, and J5-3.** Mucous gel covers the epithelial cell layer of the large intestine (3), and *E. coli* has been observed in close association with the mucous secreting brush border cells of the human colon (9). With this in mind, the following experiment was performed. *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 ( $2.4 \times 10^9$  cells each) were incubated over a range of concentrations with

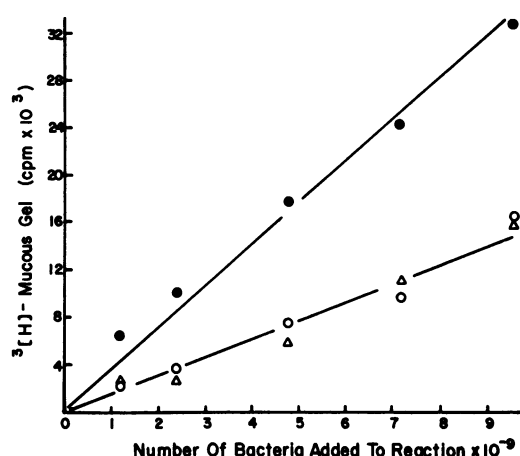


FIG. 3. Binding of <sup>3</sup>H-labeled mucous gel to *E. coli*. Experiments were performed as described in the text. Symbols: (●) F-18; (○) F-18 Col<sup>-</sup>; (Δ) J5-3.

<sup>3</sup>H-labeled mucous gel (72 μg,  $9 \times 10^5$  cpm) in a total volume of 0.1 ml for 1 h at 37°C. At that time, the amounts of mucous gel bound to each strain were determined (Fig. 3). The amount of mucous gel bound to each strain was directly proportional to the bacterial concentration; however, strain F-18 bound two to three times as much mucous gel per cell as did either strain F-18 Col<sup>-</sup> or J5-3. That is, the relative mucous gel binding ability of each strain (Fig. 2) reflected its relative colonizing ability (Fig. 1).

An experiment was also performed to determine the relative affinities of mucous gel and a nonspecific protein, bovine serum albumin for *E. coli* F-18. <sup>3</sup>H-labeled bovine serum albumin (72 μg,  $9 \times 10^5$  cpm) and <sup>3</sup>H-labeled mucous gel (72 μg,  $9 \times 10^5$  cpm) were each incubated with *E. coli* F-18 ( $2.4 \times 10^9$  cells) in reaction mixtures of 0.1 ml for 1 h at 37°C. At that time, the amounts of <sup>3</sup>H-labeled bovine serum albumin and <sup>3</sup>H-labeled mucous gel bound by *E. coli* F-18 were determined. *E. coli* F-18 bound 10,159 cpm of <sup>3</sup>H-labeled mucous gel and 626 cpm of bovine serum albumin. Clearly, mucous gel proteins had an extremely high affinity for *E. coli* F-18 relative to that of bovine serum albumin.

**Analysis of individual mucous gel proteins bound by strains F-18, F-18 Col<sup>-</sup>, and J5-3.** Mucous gel proteins were analyzed on polyacrylamide gels (see above). Three major and at least 14 minor proteins were observed (Fig. 4). The three major proteins were designated mp-1 (26,000 daltons), mp-2 (73,000 daltons), and mp-3 (79,000 daltons).

To determine which mucous gel proteins bind to strains F-18, F-18 Col<sup>-</sup>, and J5-3, each strain ( $2.4 \times 10^{10}$  CFU) was incubated with mucous gel (720 μg of protein,  $9 \times 10^6$  cpm) in a volume

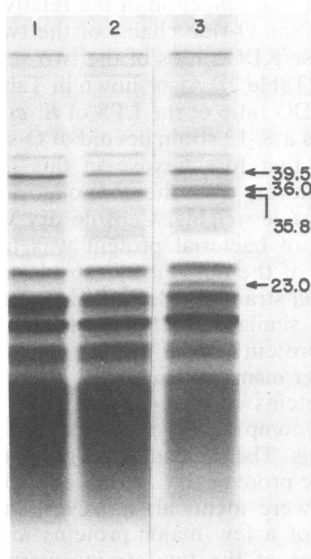


FIG. 2. *E. coli* outer membrane proteins. <sup>3</sup>H-labeled outer membrane proteins (100,000 cpm) were analyzed as described in the text. Lanes: 1, F-18; 2, F-18 Col<sup>-</sup>; 3, J5-3. The numbers beside the arrows pointing to specific proteins are in kilodaltons.

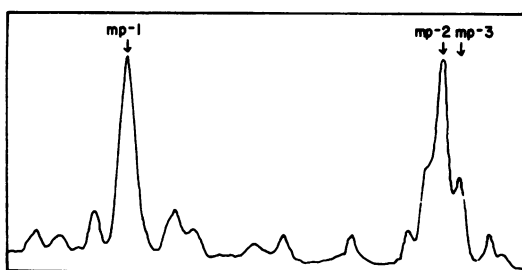


FIG. 4. Polyacrylamide gel analysis of mucous gel proteins. Mucous gel proteins (100,000 cpm) were analyzed by densitometry of fluorograms of polyacrylamide gels as described in the text.

of 1 ml, the total cell membrane fraction of each strain was isolated, and the mucous gel proteins associated with the membranes were analyzed by polyacrylamide gel electrophoresis (Fig. 5). Strain F-18 bound mp-1 almost exclusively and relatively minor amounts of the remaining proteins by comparison. In contrast, strain F-18 Col<sup>-</sup> did not bind much mp-1 relative to strain F-18, but the remaining proteins bound were in proportion to those bound by strain F-18. *E. coli* J5-3 also bound less mp-1 relative to the remaining mucous gel proteins and appeared to be similar in its mucous gel protein binding properties to F-18 Col<sup>-</sup> (Fig. 5).

**Mucous gel binding to *E. coli* LPS.** LPS ( $5 \times 10^{10}$  cell equivalents) purified from strains F-18, F-18 Col<sup>-</sup>, and J5-3 were exposed to <sup>3</sup>H-labeled mucous gel (720  $\mu$ g,  $9 \times 10^6$  cpm) in a total volume of 1 ml. *E. coli* F-18 LPS bound a total of  $6.7 \times 10^5$  cpm, *E. coli* F-18 Col<sup>-</sup> bound a total of  $6.2 \times 10^5$  cpm, and *E. coli* J5-3 bound a total of  $2.4 \times 10^5$  cpm. The proteins bound to each of the LPS extracts after centrifugation at  $100,000 \times g$  analyzed by fluorography of polyacrylamide gels (Fig. 5).

Each of the LPS preparations bound similar amounts of mp-3 per 60,000 cpm analyzed; however, in addition, strain F-18 LPS bound large amounts of mp-1 relative to either strain F-18 Col<sup>-</sup> LPS or strain J5-3 LPS (Fig. 6). These results suggest that a large part of mp-1, which binds selectively to *E. coli* F-18 whole cells (Fig. 4); binds to F-18 LPS.

**Mucous gel as a carbon and nitrogen source for *E. coli* growth.** Some members of the enteric bacteria degrade large intestine mucin (11). Since mucous gel glycoproteins are a potential source of both carbohydrate and amino acids, it was of interest to determine whether large intestine mucous gel could serve as the sole source of carbon and nitrogen for growth of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3.

Two 37°C standing cultures, each containing nonradioactive nonmethylated mucous gel (520

$\mu$ g/ml) in HEPES-Hanks buffer (pH 7.4) were inoculated to concentrations of  $5 \times 10^3$  cells per ml with each of the following pairs of *E. coli* strains: (i) F-18 and F-18 Col<sup>-</sup> and (ii) F-18 and J5-3. Generation times were determined from viable counts of each strain made at intervals after inoculation. In each culture, both strains grew with essentially identical generation times of 42 min and produced identical cell yields of about  $10^8$  CFU/ml, showing not only that mucous gel can act as the sole source of carbon and nitrogen for these strains, but also that F-18 did not inhibit the growth of either F-18 Col<sup>-</sup> or J5-3 in mixed culture (data not shown).

## DISCUSSION

*E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 each colonized streptomycin-treated mice at a level of about  $10^8$  CFU/g of feces when fed to mice individually. Therefore, each of the strains survived the complex environment of the mouse large intestine equally well. It was only when mice were fed pairs of *E. coli* strains that we were able to demonstrate that *E. coli* F-18 is a far better colonizer than either *E. coli* F-18 Col<sup>-</sup> or *E. coli* J5-3 (Fig. 1). It therefore appears that *E. coli* F-18 outcompetes the other two strains for colonization sites in the mouse large intestine.

We have found three differences in the cell

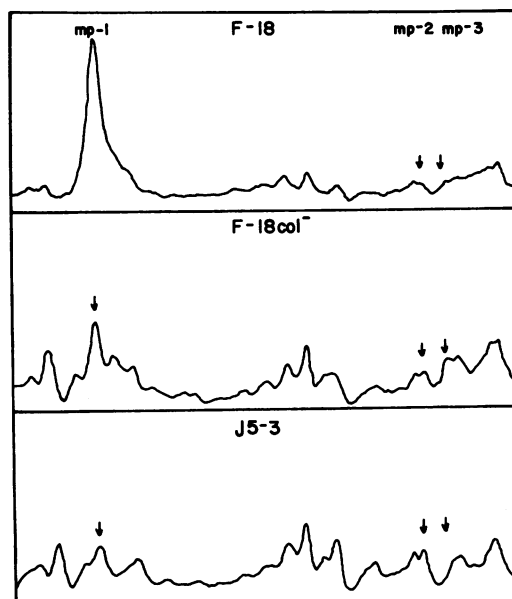


FIG. 5. Polyacrylamide gel analysis of mucous gel proteins bound to *E. coli*. Mucous gel proteins (100,000 cpm) bound to strains F-18, F-18 Col<sup>-</sup>, and J5-3 were analyzed by densitometry of fluorograms of polyacrylamide gels as described in the text.



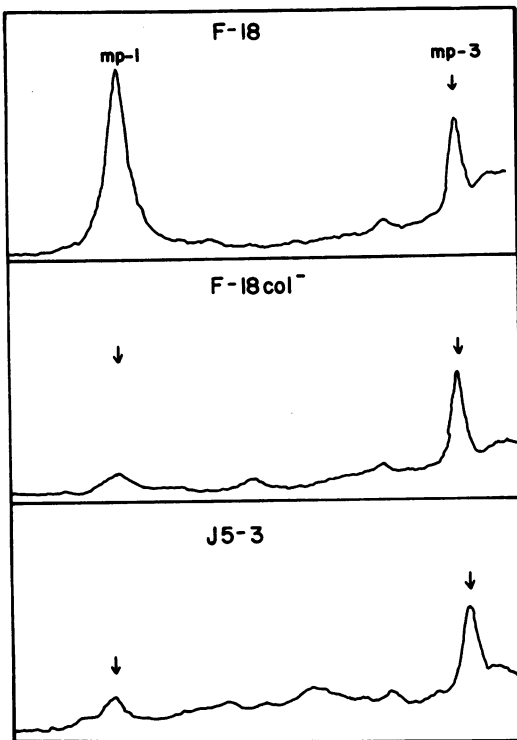


FIG. 6. Mucous gel binding to *E. coli* LPS. Mucous gel proteins (60,000 cpm) bound to LPS of *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 were analyzed by densitometry of fluorograms of polyacrylamide gels as described in the text.

surfaces of *E. coli* F-18 and *E. coli* F-18 Col<sup>-</sup>. First, although the molecular weights of the outer membrane proteins of the two strains were identical, the relative amounts of several proteins in the outer membranes of the two strains differed (Fig. 2). Second, *E. coli* F-18 was found to be less motile than *E. coli* F-18 Col<sup>-</sup>. Third, *E. coli* F-18 bound between two and three times as much mouse large intestine mucous gel as did *E. coli* F-18 Col<sup>-</sup> (Fig. 3). Much of this difference was accounted for by the ability of *E. coli* F-18 LPS to bind a single 26,000-dalton mucous gel protein (mp-1) (Fig. 6).

It might be argued that the multiple differences observed in the surfaces of *E. coli* F-18 and *E. coli* F-18 Col<sup>-</sup> are not unexpected, since the two strains differ by a  $3 \times 10^7$ -dalton plasmid and therefore by a number of genes. This explanation does not appear to be valid. We have recently isolated a second strain from *E. coli* F-18 R1<sup>+</sup>, cured of the R1 plasmid by acriflavin treatment. This strain retained all seven *E. coli* F-18 plasmids and made the F-18 colicin, but acted identically to *E. coli* F-18 Col<sup>-</sup> with respect to colonizing ability, mp-1 binding ability to whole cells and LPS, and motility. It there-

fore seems likely that the immediate parent of *E. coli* F-18 R1<sup>+</sup>, which received the R1 plasmid from *E. coli* RS-2, was a mutant with increased R1 plasmid recipient ability relative to *E. coli* F-18 (F-18 R1<sup>+</sup> transconjugants appeared at a frequency of only  $1/10^8$  recipient cells [4]). Analogous to the present situation, mutants of *E. coli* K-12 have been described, which by a single mutation were altered in LPS, outer membrane protein composition, and motility (10).

At the present time, we do not know the sequence of events leading to *E. coli* large intestine colonization, but it is clear that *E. coli* adheres to the mucous secreting brush border cells of the human colon in vivo (9). Since mucous gel covers the epithelial cells, it seems reasonable that *E. coli* penetrates the mucous gel layer to reach the epithelial cells. We have shown in this investigation that *E. coli* F-18, F-18 Col<sup>-</sup>, and J5-3 can, with equal facility, metabolize mucous gel for use as the sole source of carbon and nitrogen. Furthermore, we have shown that the ability of each strain to bind mp-1 reflects its colonizing ability (Fig. 1, 5, and 6). It is therefore possible that mp-1 is part of a mucous gel receptor for *E. coli* F-18 LPS and that the remaining mucous gel proteins serve as the source of nutrients for growth. In this way, *E. coli* F-18 daughter cells could continuously grow toward the epithelial cells by first binding to the mp-1 receptor and then metabolizing the remaining mucous gel proteins. Since *E. coli* F-18 binds mp-1 far better than does either F-18 Col<sup>-</sup> or J5-3 (Fig. 5) and also colonizes mice more efficiently than does F-18 Col<sup>-</sup> or J5-3 in cofeeding experiments (Fig. 1), it may be that *E. coli* F-18 is able to outcompete F-18 Col<sup>-</sup> and J5-3 for crucial mucous gel receptor sites. Clearly, in this system, the relative ability of an *E. coli* strain to bind mp-1 is positively correlated with its colonizing ability. It should be emphasized, however, that with some bacteria an enhanced ability to bind one or more mucous gel proteins may be negatively correlated with colonizing ability. That is, the binding of some mucous gel components by some bacteria could cause them to become trapped within the mucous gel, thereby blocking their adhesion to the mucosal surface and resulting in their elimination as the mucous gel is sloughed by peristalsis.

Finally, we do not mean to imply that mucous gel contains the final colonization sites of the large intestine. It is equally likely that the surface differences among the strains described here, or other as yet undetected surface differences, will prove to be responsible for adhesion abilities to colonic epithelial cells that may also reflect the relative colonization abilities we have observed (Fig. 1). We are presently testing this possibility.

## ACKNOWLEDGMENT

This work was supported by Public Health Service grant AI16370 from the National Institute of Allergy and Infectious Disease.

## LITERATURE CITED

- Allen, A., and D. Snary. 1972. The structure and function of gastric mucous. *Gut* 13:666-672.
- Bachmann, B. J. 1972. Pedigrees of some mutant strains of *Escherichia coli* K12. *Bacteriol. Rev.* 36:525-557.
- Clamp, J. R., G. Fraser, and A. E. Read. 1981. Study of the carbohydrate content of mucous glycoproteins from normal and diseased colons. *Clin. Sci.* 61:229-234.
- Cortiss, T. L., P. S. Cohen, and V. J. Cabelli. 1981. R-plasmid transfer to and from *Escherichia coli* strains isolated from human fecal samples. *Appl. Environ. Microbiol.* 41:959-966.
- Dische, Z. 1955. New color reactions for determination of sugars in polysaccharides, p. 313-358. *In* D. Glick (ed.), *Methods of biochemical analysis*, vol. 2. Interscience Publishers Ltd., London.
- Dufour, A. P., E. R. Strickland, and V. J. Cabelli. 1981. Membrane filter method for enumerating *Escherichia coli*. *Appl. Environ. Microbiol.* 41:959-966.
- Evans, D. G., and D. J. Evans, Jr. 1978. New surface associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. *Infect. Immun.* 21:638-647.
- Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach. 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. *Infect. Immun.* 12:656-667.
- Hartley, C. L., C. S. Newmann, and M. H. Richmond. 1979. Adhesion of commensal bacteria to the large intestine wall in humans. *Infect. Immun.* 23:128-132.
- Hazelbauer, G. L. 1977. The outer membrane and chemotaxis: Indirect influences and secondary involvements, p. 449-473. *In* M. Inouye (ed.), *Bacterial outer membranes: biogenesis and functions*. John Wiley & Sons, Inc., New York.
- Hoskins, L. C., and E. T. Boulding. 1981. Mucin degradation in human colon ecosystems. Evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes. *J. Clin. Invest.* 67:163-172.
- Isaacson, R. E., B. Nagy, and H. W. Moon. 1977. Colonization of porcine small intestine by *Escherichia coli*: colonization and adhesion factors of pig enteropathogens that lack K88. *J. Infect. Dis.* 135:531-539.
- Jann, K., B. Jann, G. Schmidt, E. Blumenstoch, and K. Vosbeck. 1981. Adherence of *Escherichia coli* to mammalian and yeast cells. The role of piliation and surface hydrophobicity. *Infect. Immun.* 32:484-489.
- Jentoft, N., and D. G. Dearborn. 1979. Labeling of proteins by reductive methylation using sodium cyanoborohydride. *J. Biol. Chem.* 254:4359-4365.
- Jones, G. W., and J. M. Rutter. 1974. The association of K88 with hemagglutinating activity in porcine strains of *Escherichia coli*. *J. Gen. Microbiol.* 84:135-144.
- Karkhanis, Y. D., J. Y. Zeltner, J. J. Jackson, and D. J. Carlo. 1978. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of gram-negative bacteria. *Anal. Biochem.* 85:595-601.
- Komeda, Y., T. Ichio, and I. Tetsuo. 1977. Effects of gal U mutation on flagellar formation in *Escherichia coli*. *J. Bacteriol.* 129:908-915.
- Laskey, R. A., and D. A. Mills. 1975. Quantitative film detection of  $^3\text{H}$  and  $^{14}\text{C}$  in polyacrylamide gels by fluorography. *Eur. J. Biochem.* 56:335-341.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Lynch, K. R., D. Pennica, H. L. Ennis, and P. S. Cohen. 1979. Separation and purification of the mRNAs for vesicular stomatitis virus NS and M proteins. *Virology* 98:251-254.
- Myhal, M. L., D. C. Laux, and P. S. Cohen. 1982. Relative colonizing abilities of human fecal and K12 strains of *Escherichia coli* in the large intestines of streptomycin-treated mice. *Eur. J. Clin. Microbiol.* 1:186-192.
- Nikaido, H. 1973. Biosynthesis and assembly of lipopolysaccharide and the outer membrane of gram-negative cell wall, p. 131-208. *In* L. Leive (ed.), *Bacterial membranes and walls*, vol. 1. Marcel Dekker, Inc., New York.
- Orskov, I., F. Orskov, H. W. Smith, and W. J. Sojka. 1975. The establishment of K99, a thermolabile, transmissible, *Escherichia coli* K antigen, previously called "K<sub>co</sub>", possessed by calf and lamb enteropathogenic strains. *Acta Pathol. Scand. Sect. B* 83:31-36.
- Revel, H. R. 1966. Restriction of nonglucoylated T-even bacteriophage: properties of permissive mutants of *Escherichia coli* B and K12. *Virology* 31:688-701.
- Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.* 31:107-133.
- Schnaitman, C. A. 1974. Outer membrane proteins *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* O111 outer membrane consists of four distinct polypeptide species. *J. Bacteriol.* 118:442-453.
- Vosbeck, K., H. Handschin, E. B. Menge, and O. Zak. 1979. Effects of subminimal inhibitory concentrations of antibiotics on adhesiveness of *Escherichia coli* *in vitro*. *Rev. Infect. Dis.* 1:845-851.
- Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides. Extractions with phenol-water and further applications of the procedure, p. 83-91. *In* R. L. Whistler (ed.), *Methods in carbohydrate chemistry*, vol. 5. Academic Press, Inc., New York.